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Genotypic Analyses of *Escherichia coli* O157:H7 and O157 Nonmotile Isolates Recovered from Beef Cattle and Carcasses at Processing Plants in the Midwestern States of the United States

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***Escherichia coli* O157:H7 and O157 nonmotile isolates (*E. coli* O157) previously were recovered from feces, hides, and carcasses at four large Midwestern beef processing plants (R. O. Elder, J. E. Keen, G. R. Siragusa, G. A. Barkocy-Gallagher, M. Koohmaraie, and W. W. Laegreid, Proc. Natl. Acad. Sci. USA 97:2999–3003, 2000). The study implied relationships between cattle infection and carcass contamination within single-source lots as well as between preevisceration and postprocessing carcass contamination, based on prevalence. These relationships now have been verified based on identification of isolates by genomic fingerprinting. *E. coli* O157 isolates from all positive samples were analyzed by pulsed-field gel electrophoresis of genomic DNA after digestion with *Xba*I. Seventy-seven individual subtypes (fingerprint patterns) grouping into 47 types were discerned among 343 isolates. Comparison of the fingerprint patterns revealed three clusters of isolates, two of which were closely related to each other. Remarkably, isolates carrying both Shiga toxin genes and nonmotile isolates largely fell into specific clusters. Within lots analyzed, 68.2% of the postharvest (carcass) isolates matched preharvest (animal) isolates. For individual carcasses, 65.3 and 66.7% of the isolates recovered postevisceration and in the cooler, respectively, matched those recovered preevisceration. Multiple isolates were analyzed from some carcass samples and were found to include strains with different genotypes. This study suggests that most *E. coli* O157 carcass contamination originates from animals within the same lot and not from cross-contamination between lots. In addition, the data demonstrate that most carcass contamination occurs very early during processing.**

Escherichia coli O157:H7 or O157 nonmotile (both referred to herein as *E. coli* O157) are classified as enterohemorrhagic *E. coli* and can cause diseases ranging in severity from nonbloody diarrhea (46) to hemolytic-uremic syndrome and death. Several factors have been associated with *E. coli* O157 virulence, including production of at least one of two Shiga toxins, intimin, and enterohemolysin (37, 41). *E. coli* O157:H7 has been declared an adulterant in ground beef due to frequent association of disease with consumption of undercooked hamburgers (26, 49). The organism commonly is present in cattle feces, suggesting that the animal is the source of beef contamination.

Studies clarifying the direct role of animal infection in subsequent carcass contamination, as well as the frequency of cross-contamination, have been limited (12, 13). A few reports have suggested that hides are a significant source of bacterial carcass contamination (6, 10, 34, 44). Most reports have approached the problem by examining the potential for carcass contamination at critical processing steps by following changes in total aerobic, coliform, or generic *E. coli* counts or some combination of these (6, 17–19). These measurements can imply causality but lack the ability to directly link sources with the introduction of specific organisms (contamination events). Pulsed-field gel electrophoresis (PFGE) genotyping has been used to track sources of *Listeria* contamination (20), and commonly is used by the Centers for Disease Control and Prevention and others to track sources of *E. coli* O157 outbreaks (e.g., see references 2, 5, 7, 24, and 42). However, prior to this study it had not been used to track *E. coli* O157 contamination of carcasses.

Previously, we reported the preharvest and postharvest prevalence of *E. coli* O157 at four large, Midwestern processing plants during July and August (13). Samples were taken from animals (preharvest) and carcasses (postharvest) within the same lot, but not necessarily from the same animals. A correlation was noted between the prevalence of *E. coli* O157 found preharvest and postharvest. Furthermore, carcasses found contaminated in the cooler also were found to be contaminated preevisceration. These correlations suggested relationships (i) between isolates entering the plant with animals and those that appear on carcasses within the same lot and (ii) between isolates found on carcasses in the cooler and those found on the carcass earlier in processing. However, in the absence of specific identification of the isolates these relationships could not be confirmed. The recovered *E. coli* O157 isolates have now been characterized by *Xba*I PFGE genotyping. The data have been used to track and confirm the sources of carcass contam-

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ination throughout processing and to examine isolate relatedness along with genotypic variability.

MATERIALS AND METHODS

***E. coli* O157 isolates.** The recovery of *E. coli* O157 isolates from cattle feces, hides, and carcasses has been described previously (13). Within each single-source lot, different samples were not necessarily taken from the same animals, but each individual carcass was tracked and sampled preevisceration, postevisceration, and postprocessing (final sampling, in the cooler). Lots ranged in size from 35 to 85 animals, and 20% of each lot was sampled. The characterization of one isolate per positive sample was described in regards to biochemical and immunological analyses, the presence of toxin and other virulence genes, and motility (13). At the time, up to three additional *E. coli* O157 isolates from postharvest samples also were recovered and stored from additional, randomly chosen, morphologically correct colonies picked from selective plates after enrichment and immunomagnetic separation. Some of these secondary isolates have now been characterized by the same methods.

Genomic fingerprint analyses. *E. coli.* O157 isolate fingerprints generated and analyzed in this study were based on PFGE separation of *Xba*I-digested genomic DNA as previously described (11); this is the method used by members of PulseNet (http://www.cdc.gov/ncidod/dbmd/pulsenet/pulsenet.htm). Pulsed-field gel certified agarose was obtained from Bio-Rad (Hercules, Calif.); Tris-borate-EDTA running buffer and lysozyme were purchased from Sigma (St. Louis, Mo.). *Xba*I and *Rsa*I were purchased from New England Biolabs (Beverly, Mass.), and *Taq* polymerase was purchased from Promega (Madison, Wis.). Lambda concatemers (Bio-Rad) were used as size markers. *E. coli* O157 strain G5244 (Bio-Rad) was used as a control and for standardization of gels. Banding patterns were analyzed and comparisons made using Molecular Analyst Fingerprinting software (Bio-Rad), employing the Dice similarity coefficient in conjunction with the unweighted pair group method using arithmetic averages (UPGMA) for clustering. Isolates were grouped into types that likely had the same origin based on fingerprint pattern similarities. Types were defined strictly as isolates that grouped together and had one; one and two; or one, two, and three band differences among their fingerprints (approximately >95% Dice similarity) (Fig. 1). Isolates with two or three band differences in their fingerprints, but not grouping with isolates that had a one-band difference, were classified as distinct types. Subtypes were defined as isolates with identical fingerprint patterns.

Genomic fingerprint stability. Fourteen isolates were selected for analysis of genome stability. These isolates included pairs from types 23, 4, 6, and 1, each with fingerprints differing by one band (Fig. 1). Also included were three isolates each from types 39 and 46; each group included two identical isolates from large subtypes and one isolate with a one-band difference. Cells were recovered from frozen (−70°C) stocks and heavily streaked onto Trypticase soy agar (TSA) (Difco Laboratories, Detroit, Mich.), followed by overnight incubation at 37°C. This was considered the day 1 culture. An isolated colony was subsequently subcultured (passaged) daily, alternating TSA and sorbitol MacConkey's agar supplemented with cefixime (0.5 mg/liter) and potassium tellurite (2.5 mg/liter; Dynal, Lake Success, N.Y.) (ctSMAC). At days 1, 5, 10, and 15 genomic DNA fingerprints were prepared as described above. In addition, cells from the same isolated colony subcultured from TSA to ctSMAC on day 1 were transferred to 3 ml of brilliant green bile broth (Difco) and passaged daily at 37°C. Genomic DNA fingerprints were prepared from these broth cultures at day 3.

PCR-restriction fragment length polymorphism analysis. The presence of the H7 gene was detected in the nonmotile isolates by the method of Fields et al. (15). In brief, approximately 1.8 kb of the *fliC* gene was amplified by PCR. Annealing temperatures were adjusted from 60 to 45°C as necessary. *Rsa*I digests of the PCR products were examined by agarose gel electrophoresis for the characteristic H7 banding pattern.

Statistical analyses. Chi-squared analyses were performed to compare frequencies of types per group using the general linear module of SAS (SAS Institute, Inc., Cary, N.C.). Results were considered significant at $P \leq 0.5$.

RESULTS

Relatedness and distribution of *E. coli* O157 isolates. One randomly selected *E. coli* O157 isolate from each positive sample recovered during a study in beef processing plants (13) was examined by *Xba*I PFGE genomic DNA fingerprinting. A total of 77 different patterns, or subtypes, were identified. The isolates divided into three main clusters (see Materials and Methods and Fig. 1). Cluster A included 76 isolates recovered from five lots during two trips to the same plant (Fig. 1 and Table 1). Clusters B and C were derived from one branch of the dendrogram and included 191 and 74 isolates, respectively (Fig. 1). A smaller group of tightly related strains within cluster B included 142 isolates (cluster B1 [Fig. 1]). Two isolates fell outside of the clusters but were more closely related to clusters B and C (types 43 and 44 [Fig. 1]).

Isolates also were typed or categorized into closely related groups as described in Materials and Methods. Forty-seven types were identified. Clusters A, B, and C included 3, 27, and 15 types, respectively (Fig. 1). Thirty-seven types included a small number of isolates and were predominately found in cluster C and in cluster B outside of B1 (65 isolates, one to five per type [Fig. 1]). Types 1, 6, and 46 accounted for 155 of the 343 isolates analyzed (45.2% [Fig. 1]). Isolates of type 1 were recovered from samples taken at all of the plants (Table 1). Isolates of types 6 and 46 were recovered from several lots sampled at individual plants (Table 1). These data do not indicate that the isolates are endemic in the plants, because they were recovered from preharvest samples in addition to postharvest samples (Fig. 1). Instead, they may represent geographically predominant strains.

The data were examined to determine if specific characteristics were associated with closely related isolates versus isolates distributed throughout the *Xba*I PFGE clusters. Isolates with different Shiga toxin profiles were distributed unevenly among the clusters; strains carrying only one *stx* gene were predominantly found among clusters A and C. These clusters included 67% of the subtypes (56% of the types) with isolates carrying only one *stx* gene, even though they accounted for just 36% of the total number of subtypes (38% of the types). These clusters also accounted for only 19% of the subtypes (22% of the types) with isolates carrying both *stx* genes. Overall, clusters A and C accounted for 81.6% of the *stx2* isolates, while cluster B included 85.9% of the *stx1 stx2* isolates (Fig. 1). Furthermore, cluster A included 52 of the 73 nonmotile isolates (71.2%), but at least one motile isolate was recovered for five of the seven subtypes in this cluster (Fig. 1). Only one nonmotile isolate grouped into cluster B1 (Fig. 1). The presence of the H7 *fliC* gene in all of the nonmotile isolates was confirmed by PCR-restriction fragment length polymorphism analysis (data not shown).

The data were further examined by chi-square analyses to determine if various characteristics were associated specifically with isolates recovered from particular types of samples (see Materials and Methods). Some bias was found between isolates from various sample sites in the *Xba*I PFGE genotype cluster, Shiga toxin profile, and motility characteristics (Table 2). Fecal isolates were underrepresented in cluster B relative to isolates from other sample sites. Compared to isolates from other sample sites, postevisceration isolates were relatively less prevalent in cluster C and more prevalent in cluster A. Also, a substantially smaller proportion of fecal isolates than carcass isolates carried both *stx1* and *stx2*. The converse was true for isolates carrying only *stx2*. Finally, a larger proportion of preharvest isolates than postharvest isolates were nonmotile.

Genomic variation among preharvest and postharvest *E. coli* O157 isolates overall. The variation in genomic fingerprints of isolates recovered both preharvest and postharvest

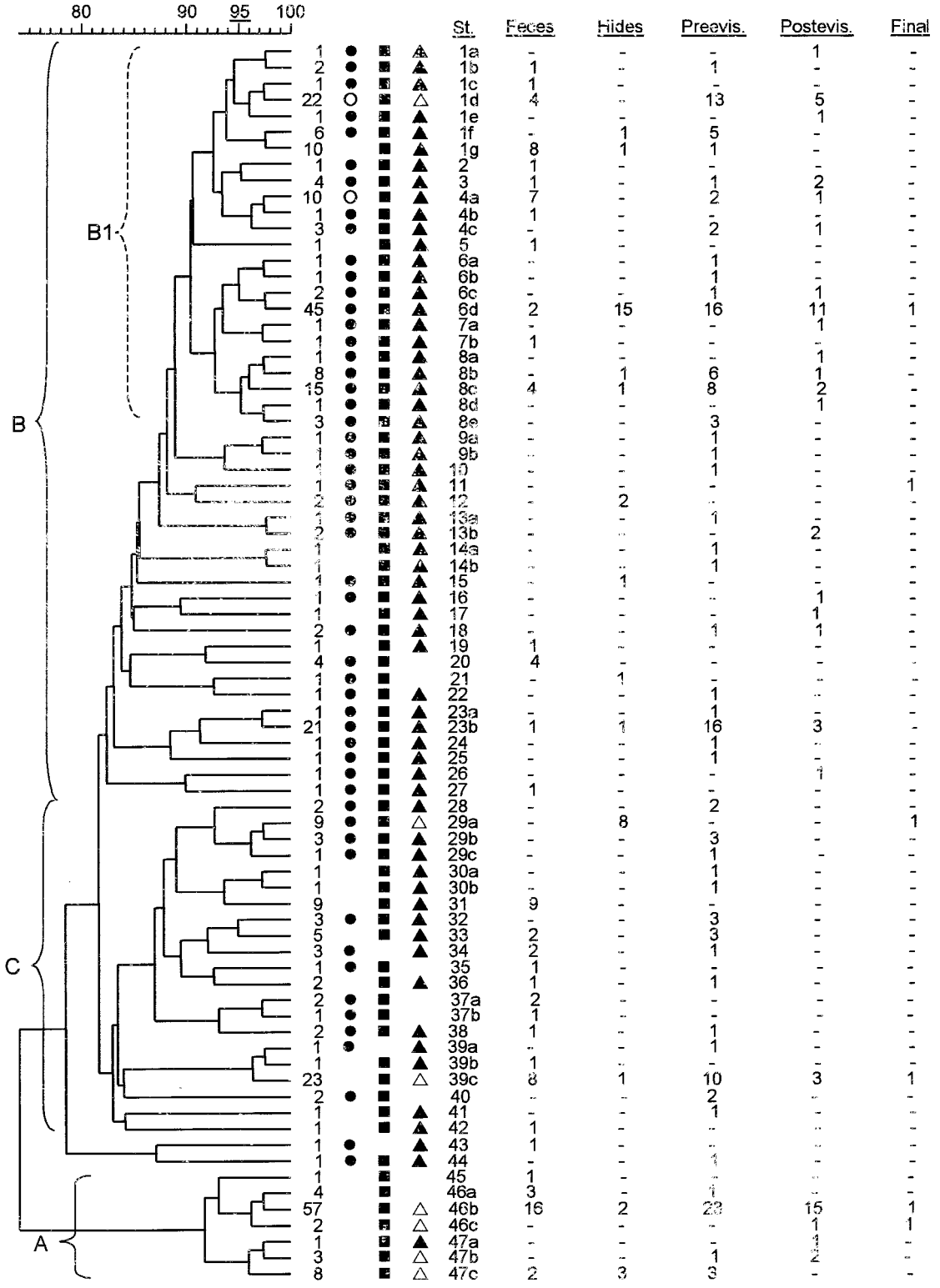

FIG. 1. Relatedness of *E. coli* O157 isolates. The dendrogram was generated using the Dice coefficient and UPGMA analysis (see Materials and Methods). The scale at the top of the dendrogram indicates the level of similarity between isolates; types include isolates connected at approximately >95% similarity. One isolate of each distinct genomic pattern (subtype) was included in the dendrogram; thus, a final branch may represent multiple isolates as indicated at the end of each branch. The presence of *stx1* is indicated by solid circles; the presence of *stx2* is indicated by solid squares. Subtypes that include isolates that carry *stx1* and *stx2* as well as isolates that carry only *stx2* are indicated by open circles and solid squares. The presence of only motile isolates is indicated by filled triangles. The presence of nonmotile and motile isolates is indicated by open triangles. The absence of a symbol indicates the absence of that characteristic from all isolates within the subtype. The numbers of isolates within each subtype that were recovered at each sampling site are indicated. Subtype designations follow the symbols. Abbreviations: St., subtype designations; Preevis., preevisceration; Postevis., postevisceration.

TABLE 1. Distribution of subtypes among lots

Lot[a]	Subtype(s)[b] recovered within cluster:				Nonclustered subtype
	B1	B (not B1)	C	A	
A1-1	1d, 1g, 4a	23b			43
A1-2		14a, 14b, 23b			
A1-3		23a, 23b	39c		
A2-1		20			
A2-2	4c				
A2-3	4c		33		
A2-4	1d	23b, 27			
B1-1					
B1-2		25			
B1-3	1d, 8a, 8b, 8e				
B1-4	1d, 7b, 8b		31		
B2-1	5, 6a, 6d		29a		
B2-2	1b, 1c, 6c, 6d, 8c	21	29a, 38		
B2-3	6b, 6d	10, 19, 26	29a		
B2-4	6d	12, 15	30a		44
C1-1	1d, 1e, 1f, 4b, 8d, 8e	11		46b	
C1-2			33, 41	45, 46a, 46b, 46c	
C1-3	1d, 1f, 7a			46b, 47a	
C1-4	1d, 4a	16	35	46b, 47b, 47c	
C2-1	8c			46a, 46b, 46c, 47b	
C2-2	3	9a, 13b, 22	37a, 37b		
C2-3	1a, 3	13b	29b, 39c		
C2-4		9b, 13a, 24	29c, 28, 34, 36 39a, 39b, 39c		
D1-1	1d		32		
D1-2	1b		32		
D1-3					
D1-4		17	42		
D2-1			39c		
D2-2	1f, 2, 8c	18	33, 40		
D2-3	1f, 8c		30b, 31		

[a] For lot designations, the letter indicates the plant, the first number indicates the trip, and the last number indicates the lot.
[b] The subtypes of *E. coli* O157 recovered from samples within each lot are indicated. Subtypes are grouped according to overall clusters (see Fig. 1).

was examined; fingerprints of both types of isolates varied substantially (Table 3). Across all lots with at least one positive sample, there was an average of one new type per 4.6 preharvest isolates and per 6.5 postharvest isolates. Thirty-two types included only preharvest or only postharvest isolates. Twenty of these types included only one isolate, and none included more than nine isolates (Fig. 1).

In order to discern the potential for the presence of multiple

TABLE 2. Distribution of phenotypic and genotypic characteristics based on subtypes, types, and sample sites

	No. (%) of:		No. (%) of isolates recovered from sample site[b] or time					No. (%) of isolates overall
	Subtypes[a]	Types[a]	Feces	Hide	Preevis.	Postevis.	Final	
Cluster								
B	47 (61)	27 (57)	39 (43)	24 (63)	88 (59)	38 (63)	2 (33)	191 (56)
C	21 (27)	15 (32)	29 (32)	9 (24)	31 (21)	3 (5)	2 (33)	74 (22)
A	7 (9)	3 (6)	22 (24)	5 (13)	28 (19)	19 (32)	2 (33)	76 (22)
stx genotype								
stx1	3 (4)	3 (6)	3 (3)	0 (0)	2 (1)	0 (0)	0 (0)	5 (1)
stx2	24 (31)	16 (34)	58 (64)	7 (18)	54 (36)	25 (42)	3 (50)	147 (43)
stx1 stx2	52 (68)	31 (66)	30 (33)	31 (82)	92 (62)	35 (58)	3 (50)	191 (56)
Motility								
Motile	69 (90)	41 (87)	63 (69)	24 (63)	128 (86)	50 (83)	5 (83)	270 (79)
Nonmotile	15 (19)	11 (23)	28 (31)	14 (37)	20 (14)	10 (17)	1 (17)	73 (21)

[a] The sum of the number of subtypes and types carrying specified *stx* genes or classified by motility is greater than the total numbers of each because some subtypes and types include isolates with more than one Shiga toxin profile or include both motile and nonmotile isolates (see Fig. 1).
[b] Abbreviations: Preevis., preevisceration; Postevis., postevisceration.

TABLE 3. Variation in *E. coli* O157 recovered at packing plants[a]

Collection time and/or site	No. of isolates	No. of types	% Unique[b]
Preharvest	129	28	14.7
Feces	91	23	16.5
Hide	38	12	10.5
Postharvest	214	34	13.6
Preevisceration	148	28	13.5
Postevisceration	60	17	13.6
Final	6	5	16.7
Overall	343	47	14.0

[a] Isolates were typed based on relatedness of *Xba*I PFGE genomic fingerprint patterns (see Materials and Methods).
[b] Percentage of unique isolates, or isolates unlike any other within the same lot.

isolate types in the samples, genomic fingerprints were generated for a group of 153 supplemental *E. coli* O157 isolates. This group was comprised of one to three additional isolates per sample (depending on availability) from 61 of the postharvest samples. The isolates were from samples in lots C1-3 and B2-2 or were from samples with an initial isolate that was (i) of a different genomic type than all preharvest isolates in the same lot or (ii) of a different genotype than another isolate(s) from a sample(s) of the same carcass taken at a different processing point(s). One or more of the additional isolates from 36 samples had a genomic fingerprint distinct from that of the initial sample isolate, although in many cases the change was by one band (data not shown). As many as three genomically distinct isolates were found within a sample. Seven new types were identified among the additional *E. coli* O157 isolates.

Genomic variation among preharvest and postharvest *E. coli* O157 isolates within lots. The diversity of *E. coli* O157 isolates within each lot was investigated. A surprisingly high number of *E. coli* O157 genomic types were recovered within each lot: as many as six types for 11 preharvest isolates and up to eight types for 15 postharvest isolates (Table 1 and data not shown). Within lots that had at least one positive sample, an average of one new type was recovered per 2.6 preharvest isolates and per 2.9 postharvest isolates. Larger lots did not necessarily include more types than smaller lots. Some of the most distantly related isolates were recovered from the same lots (e.g., lot C1-1 [Table 1]). Twenty-one lots included at least one isolate of a unique type within that lot, i.e., an isolate that was unlike any others recovered within that lot. Unique isolates were recovered proportionately from preharvest samples as well as postharvest samples (Table 2).

In order to discern a link between carriage by live animals and carcass contamination, the 17 lots with at least two preharvest and two postharvest isolates were examined by lot for a correlation between preharvest and postharvest isolate types (Table 4). Isolates of the same type were considered "a match," or "matching." Overall, within these 17 lots 68.2% (120 of 176) of the isolates recovered postharvest matched at least one preharvest isolate from the same lot. Statistical analyses of these data were not possible due to the large number of unknown variables, such as the total potential number of isolate types per sample.

Only one isolate was examined from each positive sample, leaving open the possibility that a different isolate from the same sample would match within the lot. One to three additional isolates (depending on availability) were examined from 42 postharvest samples for which the initial isolate did not match any preharvest isolate within the same lot. Isolates with different genomic types were recovered from 24 of these samples. At least one additional isolate matched a preharvest isolate from the same lot for eight of the samples, slightly increasing, to 72.7% (128 of 176), the proportion of identified matches between postharvest and preharvest isolates. Seven of the additional isolates were of unique types, i.e., unlike any other within the same lot.

Genomic variation among postharvest *E. coli* O157 isolates by carcass. The fingerprints of isolates from carcass samples taken throughout processing were compared, in order to discern if the carcass contamination found later in processing corresponded to that which was on the same carcass early in

TABLE 4. *E. coli* O157 isolate matches

Lot[a]	No. of postharvest matching preharvest[b]	Total no. of postharvest samples[c]	No. of postevisceration matching preevisceration[d]	Total no. of carcass samples[e]
A1-1	13	13	3	3
A1-2				
A1-3	5	9		
A2-1				
A2-2				
A2-3				
A2-4				
B1-1				
B1-2				
B1-3			1	2
B1-4	4	7		
B2-1	11	12	4	4
B2-2	16	17	5	7
B2-3	5	7	0	1
B2-4	1	3		
C1-1	0	8	1	2
C1-2	28	29	10	11
C1-3	5	14	2	6
C1-4	8	14	1	5
C2-1	3	5	1	1
C2-2	1	4	0	1
C2-3			0	1
C2-4	9	15	1	2
D1-1				
D1-2				
D1-3				
D1-4				
D2-1	2	2		
D2-2	9	13	3	3
D2-3	0	4		

[a] For lot designations, the letter indicates the plant, the first number indicates the trip, and the last number indicates the lot. Lots indicated in boldface type included preharvest isolates unlike any postharvest isolates.
[b] The number of postharvest samples with isolates of the same type as preharvest sample isolates. Only the 17 lots including at least two preharvest and two postharvest *E. coli* O157 positive samples were analyzed.
[c] The total number of postharvest samples testing *E. coli* O157 positive. Only the 17 lots including at least two preharvest and two postharvest *E. coli* O157 positive samples were included.
[d] The number of carcasses with matching postevisceration and preevisceration isolates. Only lots with carcasses testing positive at both sites were included.
[e] The total number of carcasses testing *E. coli* O157 positive both preevisceration and postevisceration. Only lots with carcasses testing positive at both sites were included.

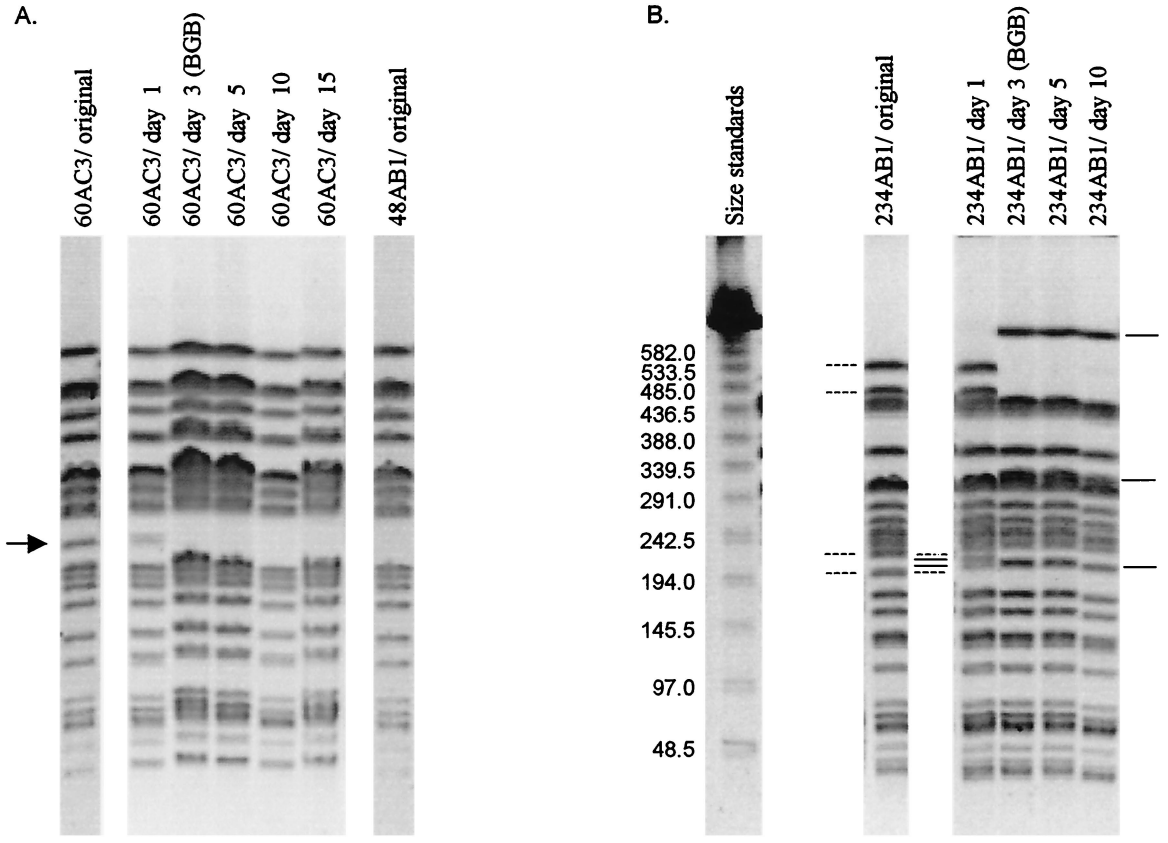

FIG. 2. Genomic fingerprints from passaged isolates. (A) Fingerprints of strain 60AC3 during passaging. The isolate and passaging day are indicated for each lane. The arrow indicates the band that was lost during passaging. (B) Fingerprints of strain 234AB1 during passaging. Size standards are lambda concatemers (Bio-Rad); sizes are given in kilobases. The isolate and passaging day are indicated for each lane. Dashed lines indicate bands lost from the original fingerprint, solid lines indicate bands gained in comparison to the original fingerprint. Bands marked between lanes 1 and 2 pertain to changes in the day 1 pattern, bands marked outside of lanes 1 and 5 pertain to changes between the original fingerprint and the day 3, 5, and 10 fingerprints. BGB, brilliant green bile broth.

processing. The initial preevisceration and postevisceration isolates examined from 32 of the 49 carcasses contaminated at both processing points (65.3%) were of the same genomic type, i.e., matched (Table 4). Additional preevisceration and/or postevisceration isolates from 15 of the carcasses were examined that did not have matching initial isolates. Matches were revealed between preevisceration and postevisceration isolates from seven more carcasses. Thus, the detected proportion of carcasses with postevisceration isolates traceable to preevisceration isolates increased to 79.6% (39 of 49). Again, statistical analyses were impossible.

Upon first analysis, four of the isolates from the six final samples positive for *E. coli* O157 matched those recovered preevisceration from the same carcass. For the remaining two carcasses, additional isolates from the positive carcass samples also did not match. However, based on the information above, it is possible that additional matches were present and simply remained undetected.

Stability of *E. coli* O157 genomic fingerprints. In order to determine the stability of the *E. coli* O157 fingerprints, 14 isolates were passaged (subcultured) as described in Materials and Methods. There was no change in the *Xba*I genomic fingerprint patterns for 12 of these isolates during passaging (data not shown).

A new *Xba*I PFGE pattern appeared during passaging of isolate 60AC3. A single band of approximately 212 kb was lost after a 3-day broth passaging or by day 5 of passaging on plates (Fig. 2A). This isolate originally was the only one of subtype 23a. The new pattern derived from 60AC3 after passaging was the same as the pattern of subtype 23b including 21 isolates. The *Xba*I PFGE pattern of isolate 48AB1, of subtype 23b, did not change during passaging (data not shown).

After 1 day of passaging, the fingerprint from isolate 234AB1 (subtype 6c) had a four-band difference from the original pattern for that isolate; slight changes in size were noted for two bands (Fig. 2B). After further passaging in broth or on plates, a seven-band difference altogether occurred in the isolate 234AB1 fingerprints. One of the two previously altered bands was lost, as were two large bands. In addition, new bands appeared. This resulted in an apparent net loss of approximately 227 kb of DNA. The new pattern was unlike any others observed in this study and therefore constituted a new, closely related type.

DISCUSSION

***E. coli* O157 genomic variability.** Many genomic types and subtypes of *E. coli* O157 have been identified by *Xba*I PFGE

fingerprinting in this and other studies (3, 7, 14, 21, 23, 31, 43). The nature and significance of these genomic differences remain unclear. Bacteriophage have been implicated as a causative agent, and the genomic variations have been suggested to be related to the direct or indirect ability to cause disease (30). A study using multilocus enzyme electrophoresis analysis of housekeeping genes detected little difference among *E. coli* O157 strains (51), which argues that the diversity in PFGE patterns is due in large part to nonevolutionary events such as horizontal DNA transfer. However, inversions, translocations, and point mutations could have caused some of the genotypic variation (9, 47). For example, the fingerprint pattern changes that resulted from repeated subculturing of isolate 234AB1 were suggestive of inversion or translocation events, as well as a loss of large amounts of DNA. Changes in genome size may not be uncommon in *Enterobacteriaceae* (8, 50) and can be the result of duplications, deletions, and horizontal DNA transfer events associated with elements such as conjugative transposons, insertion elements, and lysogenic bacteriophage (9, 47). Although duplications may not be unlikely in *E. coli* (22), numerous studies have suggested that horizontal DNA transfer by a variety of mechanisms occurs in and across many bacterial species in vivo (16, 25, 27, 35, 38–40, 48). Rode et al. (45) found that a sepsis-associated strain of *E. coli* and a uropathogenic strain of *E. coli* had distinct deletion and insertion events associated with novel DNA in comparison to *E. coli* K-12 rather than genomic rearrangements, which suggests horizontal DNA transfer events had occurred. DNA transfer by the *E. coli* O157 Shiga toxin phages in vivo has been reported (1), and even the *E. coli* K-12 genome contains evidence of substantial horizontal or lateral DNA transfer (8, 32).

This study is the first to report that motility as well as carriage of *stx* genes corresponded to specific genotypic clusters. The only similar observation was made by Karch et al. (28), who reported a group of distinct patterns for sorbitol-fermenting *E. coli* O157:H$^-$ isolates. For the most part, the nonmotile isolates recovered in this study did have *Xba*I PFGE genotypes identical to those of some motile, H7$^+$ isolates. These nonmotile isolates may have undetected mutations or may simply prefer different conditions to stimulate expression of the H7 antigen and motility, such as passaging in semisolid media. Reports differ on the correlation of *E. coli* O157 Shiga toxin profiles with subtypes (33, 43). The data reported here demonstrated a strong association between Shiga toxin profile and *Xba*I PFGE subtype, although two subtypes included both *stx2* and *stx1 stx2* isolates (subtypes 1d and 4a [Fig. 1]). The absence of *stx1* in the *stx2* isolates was confirmed by colony blotting and an additional PCR procedure (data not shown). Murase et al. (36) noted by a different PFGE technique that loss of an ~70-kb band corresponded to loss of either *stx* gene. It is possible that the presence or absence of a band this size was not clearly distinguished in these analyses. Alternatively, other phage may be present in the strains lacking *stx1*, such that their size and position masked the absence of an *stx1* phage.

Two or three main *E. coli* O157 relatedness clusters have been found in this and other studies by various genomic analyses (30, 33, 51). It has been suggested that genomic variation is related to the ability of the organism to cause disease (30). It is possible that each cluster consists of one or a few core genotypes that are primarily altered by independent horizontal DNA transfer events resulting in the multitude of subtypes and types. The detection of a few predominant genotypes and many less populous genotypes in this and other studies is in keeping with this hypothesis (31, 43). The derivation of several genotypes from a single genotype during in vivo passaging of *E. coli* O157 has been observed, although the causes of the alterations were not determined (4, 29). The derivation of two new genotypes from a single *E. coli* O157 isolate was also observed during in vitro passaging in this study.

Up to four types or subtypes of *E. coli* O157 were found per lot in cattle feces (data not shown). This observation is similar to the results of previous studies (31, 43). Rice et al. also (43) reported no relationship between the number of subtypes and the number of samples per farm. The number of preharvest *E. coli* O157 *Xba*I PFGE types recovered per lot increased to as many as six when hide isolates were added to the analysis. Therefore, the cattle may have actually carried or been exposed to more types of *E. coli* O157 than those recovered from feces. Exposure of hides to *E. coli* O157 in the feces of wild animals, potential difficulties recovering all possible types from different sources (i.e., feces or hides), or the possible inability of all *E. coli* O157 types to survive under various conditions could account for the extra variation.

Tracking of *E. coli* O157 carcass contamination. Chapman et al. (12) studied *E. coli* O157 contamination of carcasses at a South Yorkshire abattoir and found that 30% of the carcasses (seven carcasses) from cattle with feces positive for *E. coli* O157 were contaminated, and 8% of the adjacent carcasses (two carcasses) also were contaminated. Direct contamination and cross-contamination were implicated by phage typing and plasmid profiles of the strains. In addition, Byrne et al. (10) showed that spreading *E. coli* O157:H7-inoculated feces onto hides resulted in contamination of the carcass and workers' hands and knives. Because of the relatively low number of *E. coli* O157-positive hides detected, the data from this study were insufficient to provide evidence that either hides or feces were more likely to be the direct source of *E. coli* O157 on the carcasses. The data did clearly demonstrate a strong relationship between preharvest and postharvest isolates within a lot, corroborating the previous observation of an overall positive correlation between preharvest and postharvest prevalence by lot (13). The isolate in vitro-passaging data suggest that more of the carcass isolates could have originated from preharvest isolates within the same lot, but the matches were not identified because of genomic alterations between individual sample isolations that were sufficient to change the designated *Xba*I PFGE type. Expanding the typing limitations may have revealed additional valid matches but likely also would have misidentified matches not reflective of the actual source of contamination. In addition, the ratios of types to numbers of isolates suggest that if additional preharvest isolates had been recovered, more potentially matching types might have been found.

The presence of more types of *E. coli* O157 in postharvest samples than in preharvest samples suggests that additional types of *E. coli* O157 were present preharvest and were not identified in this study. Since a proportionate number of unique isolates were recovered preharvest as well as postharvest, and a proportionate number of types were unique to preharvest isolates and postharvest isolates, the data do not

suggest cross-contamination. Cross-contamination of the carcasses presumably would be due to animals within lots entering the plant earlier in the day. Cross-contamination previously was suggested as the source of *E. coli* O157 on carcasses within lots that did not include positive preharvest samples (13). When the *Xba*I PFGE genotypes were examined with regard to matches between postharvest and preharvest isolates across lots, the data were found to be inconclusive. In some cases preharvest (animal) isolates matched postharvest (carcass) isolates from an earlier lot, and in several instances preharvest isolates from different lots were of the same type (data not shown). Therefore, cross-contamination between lots could not be discerned. However, for each trip to a plant, there was no evidence of cross-contamination worsening over the course of the sampling period. The numbers of matching preharvest and postharvest isolates did not decrease for lots processed later in the day, and carcasses from later lots were not more frequently contaminated than carcasses from lots processed earlier in the day. The latter observations need to be interpreted with caution, though, as the study was not designed to compare the data in this manner. Overall, while cross-contamination probably occurred to a limited extent, some of the prevalence data may have been reflective of a higher difficulty in recovering *E. coli* O157 from preharvest sources. Competing microflora in fecal and hide samples necessitates the use of more-stringent enrichment conditions for these samples compared to carcass samples (13).

For many postharvest samples, more than one genomic type of *E. coli* O157 was recovered. These data could suggest that individual carcass contamination originated from multiple sources or that contamination sources (feces and hide) may harbor multiple isolates that can be transferred to the carcass in a single contamination event. Work identifying *E. coli* O157 of multiple genomic types in individual cattle feces supports the latter interpretation (31). Only one isolate was examined per preharvest sample in this study, so additional types may have been present in hide or fecal samples and remained undetected.

Further studies are needed to detail the potential for carcass-to-carcass cross-contamination. We did not sample adjacent carcasses, so the potential for direct cross-contamination was not discerned. The clear majority of carcass contamination with *E. coli* O157 occurred prior to any direct contact between carcasses (13), although cross-contamination during very early processing steps via equipment and workers could have occurred (10). Regarding potential cross-contamination later in processing, most of the carcasses found to be contaminated later in processing had been contaminated earlier in processing (49 of 59 [reference 13]). In addition, the PFGE patterns of most isolates from the later processing samples matched those of isolates recovered from the same carcass earlier in processing. Therefore, *E. coli* O157 contamination found later in processing was due largely to contamination that occurred early in processing and not to carcass-to-carcass contamination.

Several additional and particularly interesting observations were made during this study. First, postevisceration isolates were underrepresented in cluster C. Second, predominant preharvest and postharvest *E. coli* O157 types within a lot often were not the same (data not shown). For example in lot A1-1, one type included 12 of 15 preharvest isolates and only 2 of 13 postharvest isolates, and a second type included only 2 preharvest isolates but included 11 postharvest isolates. (The third type included one preharvest isolate.) Third, fecal isolates were less likely than isolates from other samples to group into cluster B. Fecal isolates also were less likely to carry both *stx* genes, being more likely to carry only *stx2*. Finally, compared to carcass isolates, fecal and hide isolates were slightly more likely to be nonmotile. Further experiments are necessary to examine these phenomena. The data could be evidence that some *E. coli* O157 genomic types are more successful at passing through processing steps or are more easily transferred to the carcass or could reflect the fact that different types are more easily recovered from the different sample sites based on recovery and/or enrichment methods. If these hypotheses were true, one would expect that a limited number of preevisceration types or preharvest types would be found postevisceration or postharvest, respectively. However, of the preevisceration isolates overall, 67.6% were of the same type as postevisceration isolates, and within the 17 lots used to analyze preharvest/postharvest isolate relatedness, 72.1% of the preharvest isolates were of types also found postharvest.

In summary, this study tracked carcass contamination by *E. coli* O157 in processing plants within the United States. A strong association between *E. coli* O157 carried by live animals and on carcasses within the same lot was first demonstrated by prevalence data (13). *Xba*I PFGE genotyping data have further implied that *E. coli* O157 found on carcasses is primarily the result of transfer within a lot rather than cross-contamination between lots, although some cross-contamination may occur (13). Furthermore, the tracking data based on *Xba*I PFGE genotyping confirm that the majority of *E. coli* O157 found on the carcass is the result of preevisceration contamination, despite a dramatic reduction throughout processing in the number of carcasses contaminated (13). Taken together, these data indicate the need to apply additional in-plant intervention strategies aimed at preventing direct contamination of the carcasses early in processing. In addition, a substantial level of genomic variation was observed among the *E. coli* O157 isolates recovered during this study. The significance of this divergence remains to be seen, although it has been implied to affect, either directly or indirectly, the ability of the organism to cause disease in humans (30). Work is in progress to investigate the relationships between these genomic differences and various aspects of the organism's ability to eventually cause disease, such as relative virulence or survival during processing, storage, and cooking.

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